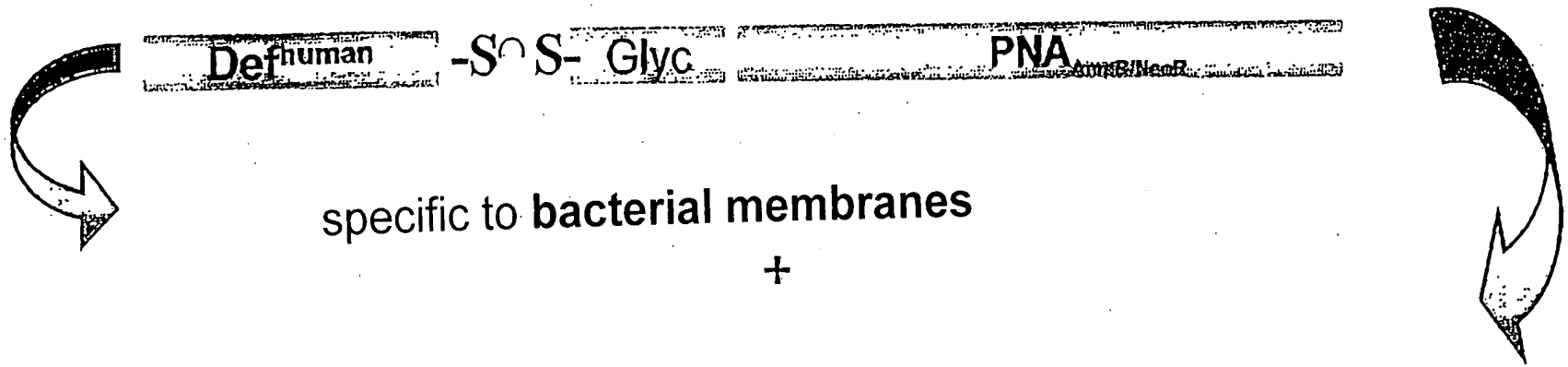
Strategies:



specific to **bacterial membranes**

+

anti-gene strategy directed specifically against **bacterial genes**

→ combined 'dual strategy'

Fig. 1

Genome site of action – anti-gene PNA

DEFINITION Cloning vector pBR322, complete genome.
ACCESSION J01749 - Genbank
KEYWORDS ampicillin resistance; beta-lactamase; cloning vector; drug resistance protein; origin of replication; plasmid;
SOURCE Cloning vector pBR322.
ORGANISM Cloning vector pBR322
artificial sequence; vectors.

```
TTCTCATGTT TGACAGCTTA TCATCGATAA GCTTTAATGC GGTAGTTTAT CACAGTTAAA      60

TTGCTAACGC AGTCAGGCAC CGTGTATGAA ATCTAACAAT GCGCTCATCG TCATCCTCGG     120

CACCGTCACC CTGGATGCTG TAGGCATAGG CTTGGTTATG CCGGTACTGC CGGGCCTCTT     180
GCGGGATATC GTCCATTCCG ACAGCATCGC CAGTCACTAT GGCGTGCTGC TAGCGCTATA     240
TGCGTTGATG CAATTTCTAT GCGCACCCGT TCTCGGAGCA CTGTCCGACC GCTTTGGCCG     300
CCGCCCAGTC CTGCTCGCTT CGCTACTTGG AGCCACTATC GACTACGCGA TCATGGCGAC     360
CACACCCGTC CTGTGGATCC TCTACGCCGG ACGCATCGTG GCCGGCATCA CCGGCGCCAC     420
AGGTGCGGTT GCTGGCGCCT ATATCGCCGA CATCACCGAT GGGGAAGATC GGGCTCGCCA     480
CTTCGGGCTC ATGAGCGCTT GTTTCGGCGT GGGTATGGTG GCAGGCCCCG TGGCCGGGGG     540
ACTGTTGGGC GCCATCTCCT TGCATGCACC ATTCCTTGCG GCGGCGGTGC TCAACGGCCT     600
```

anti-gene PNA

HOOC–TAC TTT AGA TTG TTA–NH_2

Fig. 2

Alignments **Holin-Protein** (Phagen) - ***Transportprotein***

product="probable holin" (GMSE-1)
[Endosymbiont bacteriophage may influence susceptibility to trypanosome infection in tsetse, Dale and Young]
protein_id="AAG50251.1"
db_xref="GI:12276078"
translation="MPCLIHLVGWGSSPGSALIREQAIGAGLAAWMTCLRGRYLGRGWRKTTFDAAICALIAWF
ARDGLALVGIDNQFSYLSSIIVGYLGNDYLGALLRRRLEKKS GESNAPQ

product="holin protein" (Listeria innocua)
protein_id="CAA61518.1"
translation="MMKMEFGKELLVYMTFLVVVTPVFVQAIKKTELIPSKWLPTVSILVGAILGALATSLDGSG
SLATMIWAGALAGAGGTGLFEQFTNRAKKYGKDD

product="holin" (bacteriophage 80 alpha)
specific_host="Staphylococcus aureus RN450
function="makes hole in membrane"
protein_id="AAB39698.1"
db_xref="GI:1763242"
translation="MDINWKLRFKNKAVLTGLVGALFVFIKQVTDLFGLDLSTQLNQASAIIGAILTLLTGIGVIT
DPTSKGVSDSSIAQTYQAPRDSKKEEQQVTWKSSQDSSLTPELSAKAPKEYDTSQPFTDASNDVGFDVN
EYHHGGGDNASKIN

product="holin" (Staphylococcus bacteriophage phi 11)
note="ORF3; structural homologue of holin"
protein_id="AAA99522.1"
db_xref="GI:511841"
translation="MDINWKLRFKNKAVLTGLVGALFVFIKQVTDLFGLDLSTQLNQASAIIGAILTLLTGIGVIT
DPTSKGVSDSSIAQTYQAPRDSKKEEQQVTWKSSQDSSLTPELSAKAPKEYDTSQPFTDASNDVGFDVN
EYHHGGGDNASKIN

product="putative holin 1" (Streptococcus pneumoniae bacteriophage MM1)
function="lysis protein"
protein_id="CAC48114.1"
db_xref="GI:15074937"
translation="MKIEFFNFLRSVIQTEDGLVLYALALIVSMEIIDFVTGTIAAIINPDIEYKSKIGINGLLRKISGV
LLLMILIPASVLLPEKTGFAFLYSICLGYIAFTFQSLIENYRKLKGNVTLFQPIVKVFQRLLEKDDDTKKGE

gene="orf87a" (Streptococcus thermophilus bacteriophage Sfi21)
product="holin"
protein_id="CAA64941.1"
db_xref="GI:2292749"
translation="MKKRKKKMINFKLRLQNKATLVALISAVFLMLQQFGLHVPNNIQGINTLVGILVILGIITDP
TTKGIADSERALSYIQPLDDKEVY

gene="hol500" (Bacteriophage A500);(Listeria monocytogenes)
protein_id="CAA59363.1"
/db_xref="GI:853745"
/translation="MMKMEFGKELLVYMTFLVVVTPVFVQAIKKTELIPSKWLPTVSILVGAILGALATSLDGSG
SLATMIWAGALAGAGGTGLFEQFTNRAKKYGKDDK

product="holin" (Bacteriophage PL-1)
protein_id="BAA96748.1"
translation="MQNELLQVLAIAFVIAPITTGFTEIFKRYTPAEGKLLPVLSIGTG
ILLACVWAMAFGHLPLIGAYALAGMLSGLASVGVYQIVKPNEEVK

Fig. 3(1)

gene="lydA" (Bacteriophage P1) (enterobacteriae)
codon_start=1
product="holin"
protein_id="CAA61014.1"
db_xref="GI:974764"
translation="MLDTQELAPVAIALLLSVIGGIGTFLMDVRDGRQSGNLLGLVTEIFVAVTAGAVAYLLGQH
EGWELSITYLMVTIASNNGHEVISGMKRVNIDSILNVLTSL VKKGGGK

gene="S" (Bacteriophage H-19B)
note="similar to Bacteriophage 21 lysis gene S, encoded by GenBank Accession Number M65239" /
product="putative holin protein"
protein_id="AAD04658.1"
db_xref="GI:2668771"
translation="MEKITTGVSYTTSAVGTGYWLLQLLDKVSPSQWVAIGVLGSLLFGLLTYLTNLYFKIREDR
RKAVRGE

gene="hol" (Bacteriophage A118)
function="forms unspecific lesions into cytoplasmic membrane prior to lysis"
specific_host="Listeria monocytogenes"
note="ORF24; two products may be translated from this gene (hol-96 and hol-93)"
product="holin"
protein_id="CAB53810.1"
db_xref="GI:5823622"
translation="MIEMEFGKELLVYMTFLVVVTPVFVQAIKKTELVPSKWLPTVSILIGAILGALATFLDGSGS
LATMIWAGALAGAGGTGLFEQFTNRSKKYGEDDK

gene="Hol" (Lactobacillus casei bacteriophage A2)
product="putative holine"
protein_id="CAB87385.1"
db_xref="GI:7573220"
translation="MKINWKVAVLSVKFWLALVPAALLVVQTAAAVFGYNWDFANLGKELTAVINAVFALLTI
VGVAVDPTTEGVSDSQQALAYPALITTKAAKIKSLEDQIKALQADKAADQATSAASEVVPETSSAAPAE
SAPESVAPVASEEVK

gene="Hol" (Lactobacillus bacteriophage phig1e)
product="holin"
protein_id="CAA66751.1"
db_xref="GI:1926366"
translation="MDIITSLNLATAGELALISFFIGVIVQAIKKTGKVKNTYLPFISMGIGILAGLAAVVVTKDTN
YLNGAVAGLIVGAATSGLTDGLSVGTSAVTTAKATKDAAKTAAITQAVLNSINTTKSSDTTQVANTSN
TEGGSTSETQK

product="holin" (Lactobacillus delbrueckii subsp. lactis bacteriophage LL-H)
protein_id="AAC00556.1"
db_xref="GI:623083"
translation="MTLIDWFNLIVAIGTIALAVVASVYVHLKAKIDTKTAAGKAFDLVGKLAVWAVNEAEHSQ
DGGAAKREFAAKLISDQLKAKGITGIDEKMVYGAVETAWKEA IENVK

product="holin protein" (Lactococcus phage c2)
protein_id="AAD20611.1"
db_xref="GI:4426933"
translation="MIETLRAIGLVVFMQLLSLALEFIDTGTLKPSVRKRIAVELMVL

gene="hol" (bacteriophage phiAM2)
note="hydrophobic pore-forming protein"
product="holin"
protein_id="AAG24367.1"
db_xref="GI:10880732"
translation="MFFNNKFYNVIKWAVLTALPALSVFIGVIGKAYGWGGTDLAIITLNAFTVFLGTLAGVSAV
KYNSQPNDTKENK

Fig. 3(2)

product="holin"
protein_id="AAG24367.1"
translation="MFFNNKFYNVIKWAVLTALPALSVFIGVIGKAYGWGGTDLAIITLNAFTVFLGTLAGVSAV
KYNSQPNDTKENK

product="holin" (Bacteriophage Tuc2009)
protein_id="AAA32614.1"
db_xref="GI:496282"
translation="MNQINWKLRLKSKAFWLALLPALFLLIQAIGAPFGYKWDFVILNQQLAAVVNAAFALLAI
VGVVADPTTSGLGDSDRVLNKDKSEENK

product="holin" (Bacteriophage TPW22)
function="formation of non-specific lesions in the cytoplasmic membrane"
protein_id="AAF12704.1"
db_xref="GI:6465904"
translation="MNQINWKLRLKSKAFWLALLPALFLLIQAIGASFGYKWNFVILNQQLAAVVNAAFALLAI
VGVVADPTTSGLGDSDRVLNKDKSEENK

product="holin" (homology to Orf78 of phage HP1 and gene S of phage P21)
protein_id="AAC45168.1"
db_xref="GI:915370"
translation="MRFNMLKNSETTGAYVGSAIAIYSGFTLADWAAIFGILFGLFT M LINWYYKNK
EIKLKETALKQKIDLKEGDHE

product="holin" (Bacillus phage GA-1)
function="host cell lysis, holin formation"
protein_id="CAC21535.1"
db_xref="GI:12141291"
translation="MFEFFHSLMETDDTKVYFLLGIIGVLNIVDFFFGFINAKFNKSIAYKSSKTIDGIMRKMKFTI
MAILFIPVSVLMPEPIGLGALYVFYFGYIYAELNSILSH LKLSEDGKETEVFLDFINTFFNSTKGDKKDD

gene="hol187" (Staphylococcus phage 187)
function="forms pores to allow access of lysin to CW"
product="holin protein Hol187"
protein_id="CAA69023.1"
db_xref="GI:2764984"
translation="MLMVIMVGNVGIYLTIFLIDTGTLRHQATQEIWHGIDILKGLKC LETLLILSLNQVI

gene="s" /function="holin" (Shigella dysenteriae)
product="S protein"
protein_id="CAC05628.1"
db_xref="GI:9955825"
translation="MYQMEKITTGVSYTTSAVGMGYWFLQFLDRVSPSQWAAIGVLGSLLFGLLTYLTNLYFKI
REDRRKAARGE

gene="E"
protein_id="CAA42879.1"
db_xref="GI:14781"
db_xref="SWISS-PROT:P31280"
translation="MERWTLLDILAFLLLLSLLLPSLLIMFIPSMYKQHASLWKARSLAKTLSMASSARLTPLSSS
RTPCVLKQDSKKL

gene="xhlB" (B.subtilis DNA (28 kb PBSX/skin element region)
product="holin-like protein"
protein_id="CAA94048.1"
db_xref="GI:1225964"
db_xref="SWISS-PROT:Q99163"
translation="MNTFDKGTVIRTVLLLIALINQTMLMLGKSPLDIQEEQVNQLADALYSAGSIAFTIGTTLAA
WFKNNYVTEKGKKQRDLLRDNNLTK

Fig. 3(3)

gene="bhlA" (Bacillus subtilis 168 prophage)
product="holin-like protein"
protein_id="AAC38301.1"
db_xref="GI:2997596"
translation="MEMDITQYLSTQGPFAVLFCWLLFYVMKTSKERESKLYNQIDSQNEVLGKFSEKYDVVIE
KLDKIEQNFK

gene="bhlB" (Bacillus subtilis 168 prophage)
product="holin-like protein"
protein_id="AAC38302.1"
db_xref="GI:2997597"
translation="MFENIDKGTIVRTLLLAIALLNQIMVMLGKAAFIINEEDINHLYDCLYTIFTIVFTTSTTTAA
WFKNNYITAKGKKQKQVLKKENLFK

gene="hol" (Bacteriophage phi-Ea1h)
specific_host="Erwinia amylovora
function="pore formation"
product="holin"
protein_id="CAC17008.1"
db_xref="GI:11342496"
translation="MRKIYVVIITTIVVAGLIWAFIATQVNTGVTSKRQEDALAVSEANVGIGKEAKDQGEQATK
RADVAKEQRTHQINQLKDKLHEKAESYDSIPLSPSDVDILC RAYRSTDPVCSPTVKSD

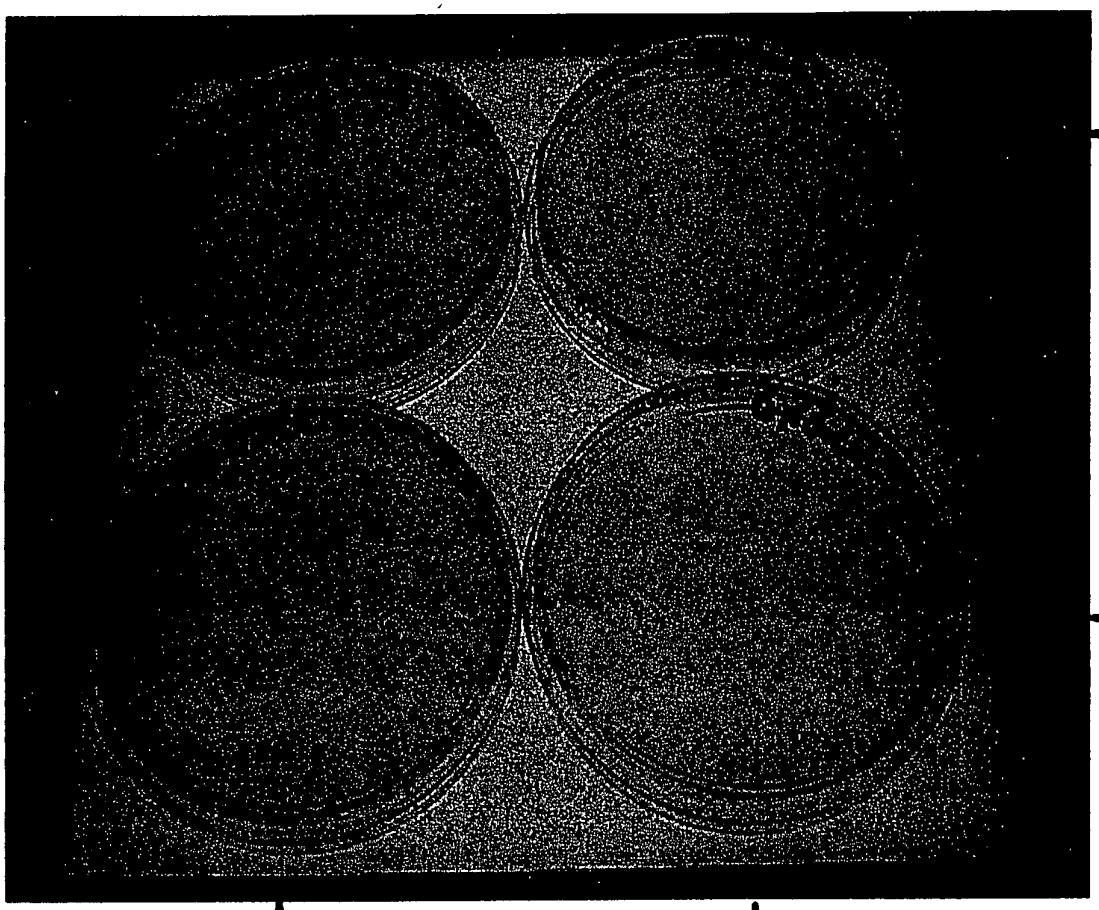
Fig. 3(4)

Alignments **Lysis-Protein** (Phagen)

product="lysis protein" (Phage phiX174)
function="host cell lysis"
protein_id="CAA84691.1"
translation="MVRWTLWDTLAFLLLLSLLLPSLLIMFIPSTFKRPVSSWKALNLRKTLLMASSVRLKPLNCS
RLPCVYAQETLTFLLTQKKTCVKNYVQKE

Fig. 3(5)

I / E. Coli$_{competent}$ XL - 10 GOLD + PNA-Probe (500 nM)



KANAMYC-Dishes

1 µl = 25 µM PNA*

AMPICILL- Dishes

10 µl = 2.5 µM PNA*

NaCl-Control

PNA* sample

Fig. 4

* peptide nucleic acid transport complex

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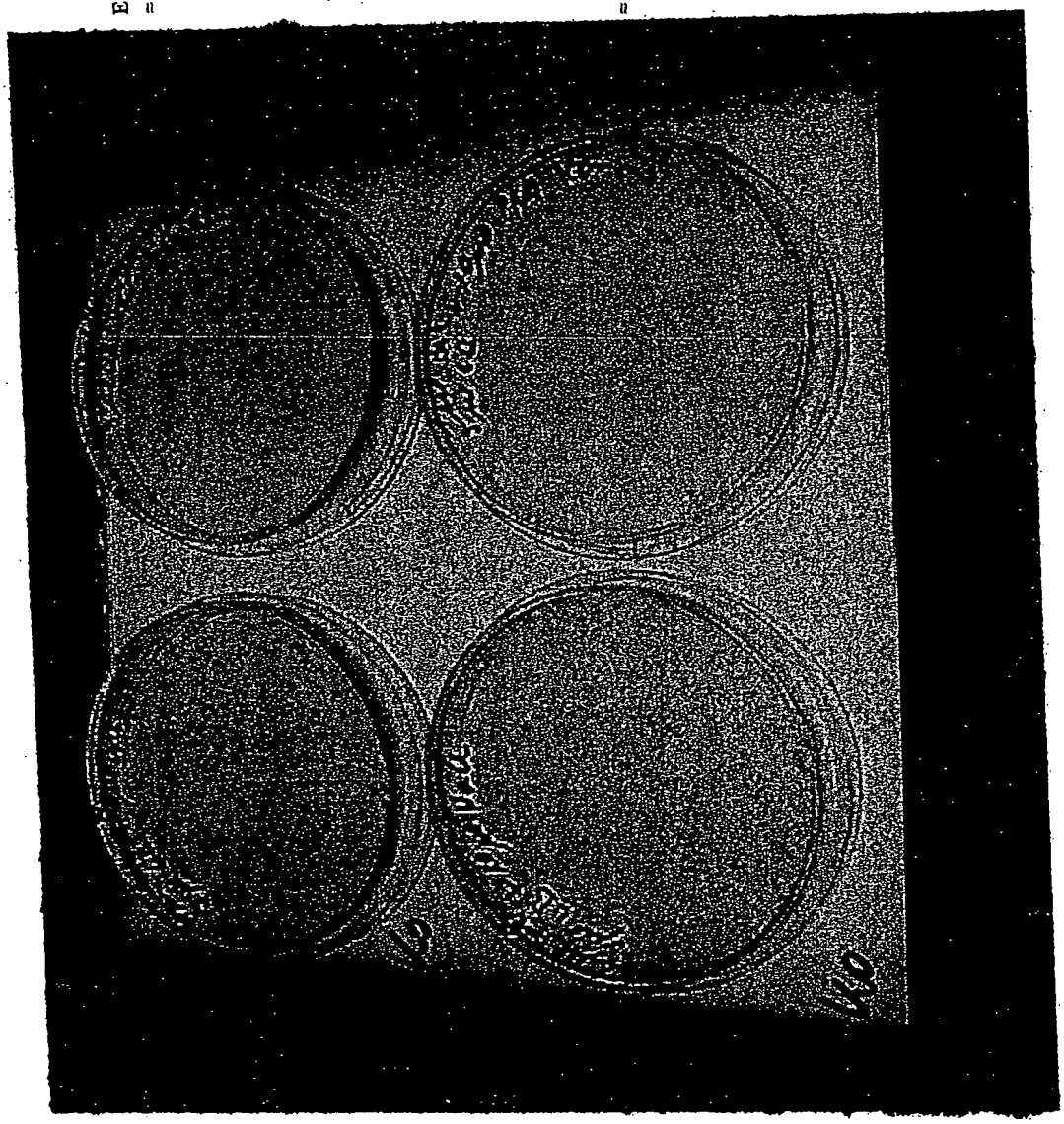
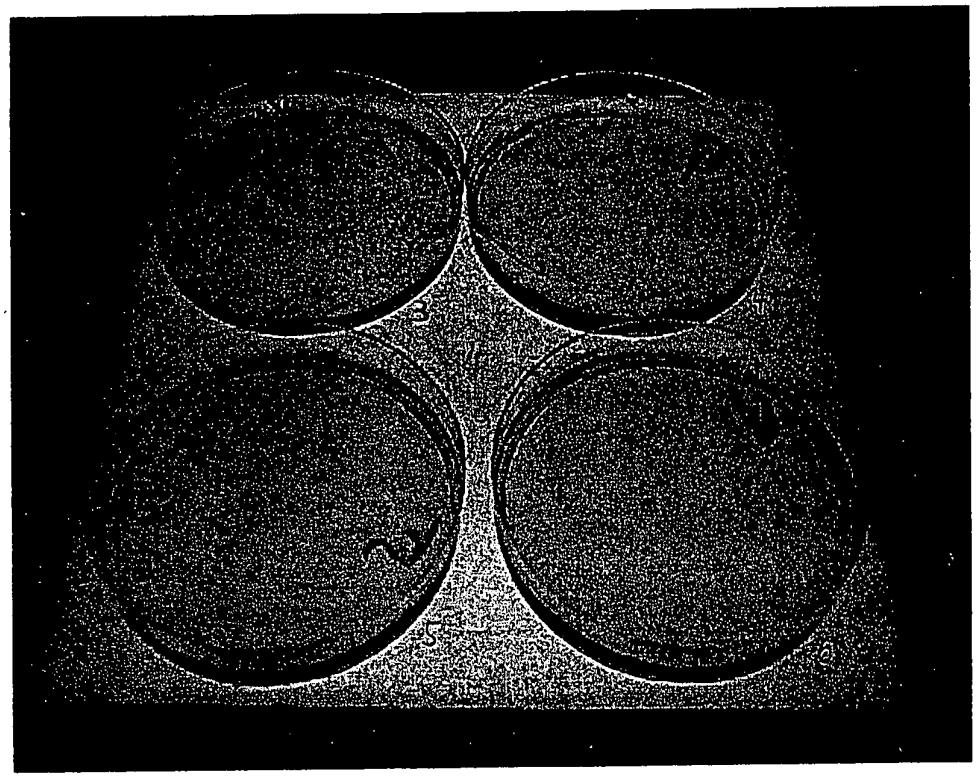


Fig. 5

E. Coli$_{intact/Kanresist}$

Optimizing Number of E. Coli per plate
serial dilution of E. Coli by spectrophotometric measurements 600 nm



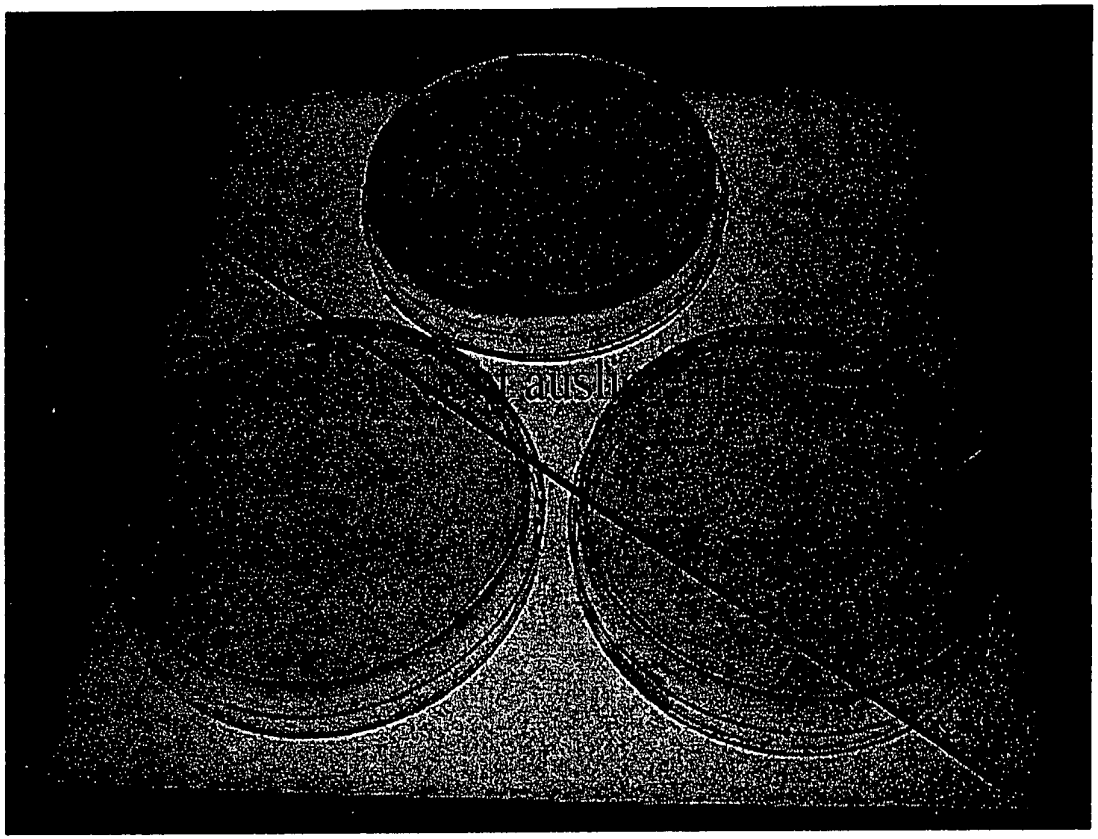
- 1) 1 : 10
- 2) 1 : 100
- 3 1: 1000
- 4) 1: 10000
- 5) 1: 100000
- 6) 1: 1000000

Serial Dilution of intact E. Coli to optimize the antibacterial incubation

Fig. 6

* peptide nucleic acid transport complex

III K1-Control E. Coli$_{intact/Kanresist}$



Kanamycine Dishes

3 PNA$_{/Ampresistgene}$ (2,5 µM) - 10 µl 2 PNA$_{/Kanresistgene}$ (25 µM) - 1 µl

Identic: Bacter. Nmb; identic. PNA conc

Fig. 7

IV / analogue III - K1-Control E. Coli$_{intact/Kanresist}$

4 PNA$_{/Ampresistgene}$ (2,5 µM) - 10 µl
1µl E. Coli + 10 10 µl PNA
in LB medium

Fig. 8

* peptide nucleic acid transport complex

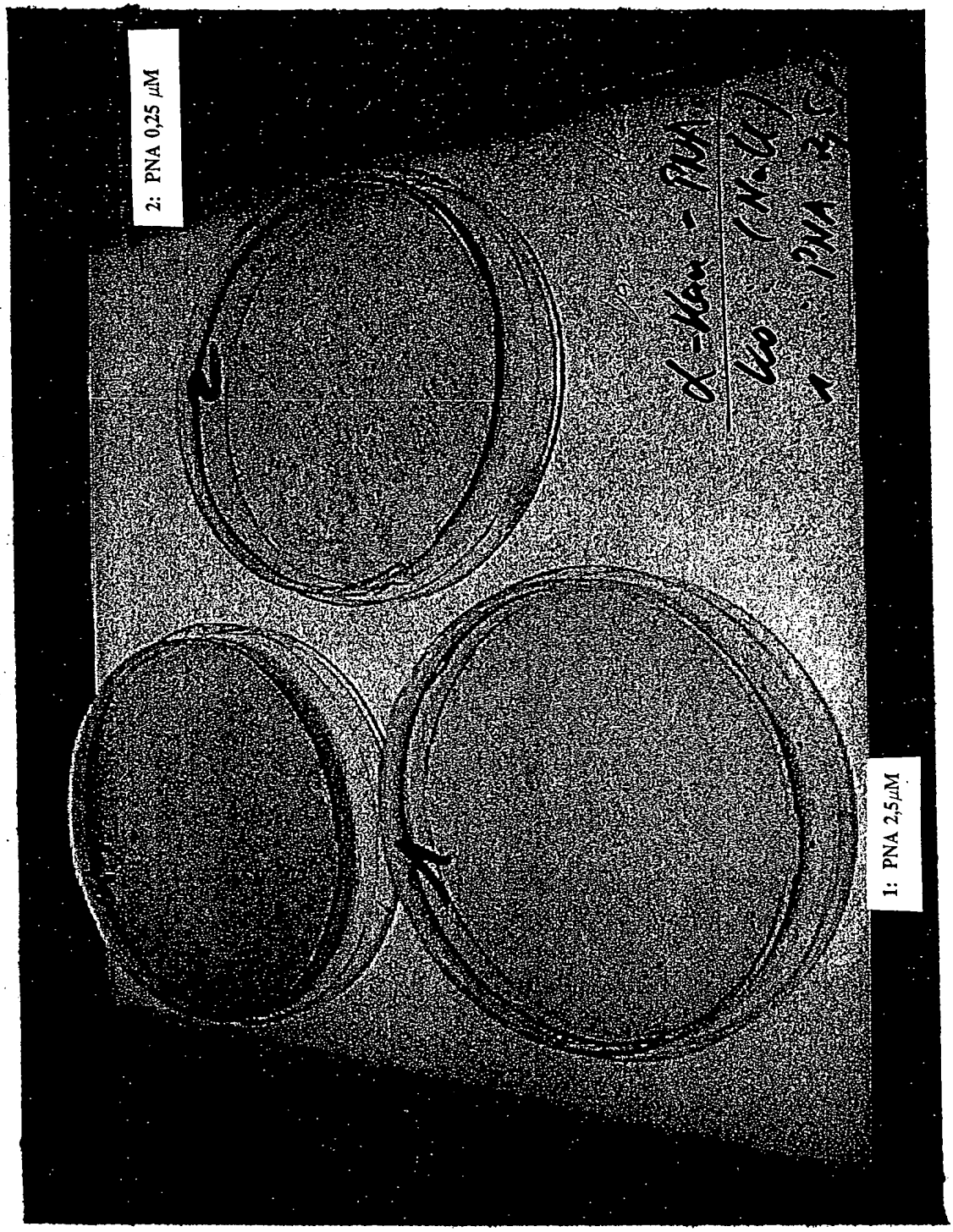
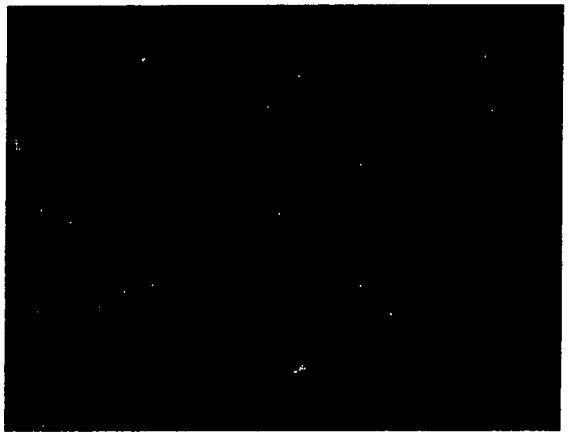


Fig. 9

Fig. 10

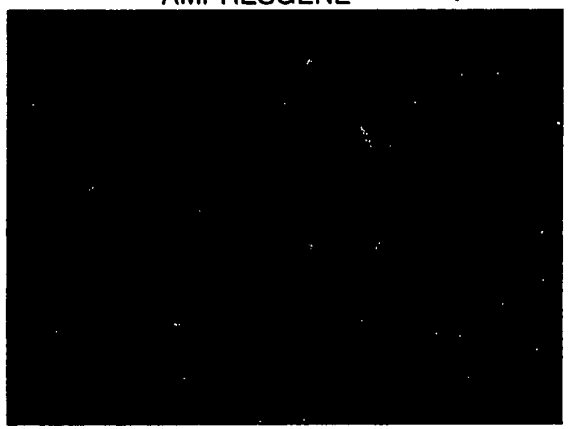
Test for NOI[illegible]-Toxicity in eucaryotic cells (HeLa)

HeLa
Control
(untreated)

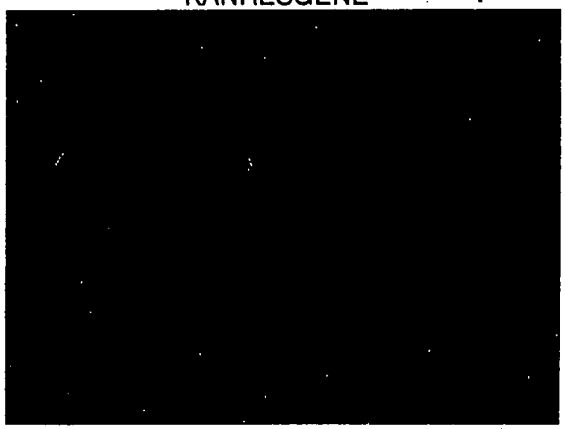


A

PNA*$_{AMPRESGENE}$ - 25 µM



PNA*$_{KANRESGENE}$ - 25 µM



B

Fig. 11

* peptide nucleic acid transport complex